Mercury Concentrations in Crayfish (*Orconectes virilis*) Tissues from Soft-water Lakes on the Canadian Shield.

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ABSTRACT

Total mercury was determined in crayfish (*Orconectes virilis*) from ten lakes located on the Precambrian Shield in south-central Ontario and from one lake in the Experimental Lakes Area, northwestern Ontario. Total mercury concentrations in abdominal flexor muscle of 124 crayfish ranged from 0.035 to 0.719 $\mu g.g^{-1}$ wet weight (carapace length: 21.5 - 48.4 mm). Size-adjusted total mercury concentrations were significantly positively correlated with lake water DOC, Fe, Al and Hg, and significantly negatively correlated with pH. Based on an examination of abdominal flexor muscle in 20 crayfish from four of the study lakes, methylmercury accounts for most (100%: SD: ± 32%) of the total mercury in crayfish muscle tissue.

Eleven tissues were analyzed for total mercury in each of 35 crayfish from six of the eleven lakes. Within each lake, mercury concentrations differed significantly among tissues ($P < 0.001$). Typical within lake relationship for multiple tissue mercury concentrations was green glands ≥ abdominal muscle = extensor muscle = claw muscle > mid and hind gut = digestive glands ≥ gastric mill = gills ≥ tergum ≥ carapace. The range in mean mercury concentrations across the six lakes for each tissue was: green glands 0.115 to 0.261, muscle tissue 0.065 to 0.144, mid and hind gut 0.039 to 0.058, digestive glands 0.031 to 0.060, gastric mill 0.024 to 0.035, gills 0.018 to 0.030 (outlier value of 0.055), eyes 0.024 to 0.034, tergum 0.014 to 0.031, and carapace 0.009 to 0.014 $\mu g.g^{-1}$ wet weight. Significant among lake differences in mercury concentrations were found for abdominal and claw muscle, green glands, gills, digestive glands, mid and hind gut, and tergum ($P \leq 0.01$). We recommend analyzing crayfish abdominal muscle to assess mercury contamination in aquatic systems; this muscle tissue, because it has a relatively

large mass and is easy to excise, appears to be a good indicator of mercury availability compared to other crayfish tissues.

INTRODUCTION

Many lakes in Ontario remote from known mercury (Hg) point sources contain predatory fish with Hg levels exceeding the Canadian 0.5 $\mu g.g^{-1}$ wet weight upper limit for safe human consumption (Suns *et al*., 1987; OMOEE & OMNR, 1993). Atmospheric deposition is believed to be a significant source of Hg to these systems (Evans, 1986; Mierle, 1990). Mercury profiles in sediment cores from several remote south-central Ontario lakes revealed that in recent decades there has been an increase in Hg inputs, possibly the result of an increase in the deposition of atmospheric Hg of anthropogenic origins (Evans, 1986). Although atmospheric deposition of Hg is considered to be a significant source of Hg to many remote Ontario lakes, it is not the total quantity of Hg in a water body that is important but rather that portion of the total which is biologically available. Mercury bioavailability is influenced by a number of physicochemical factors. For example, in relatively 'pristine' areas, water chemical parameters representative of lake acidity (pH and alkalinity) (Wren & MacCrimmon, 1983; McMurtry *et al*., 1989; Cope *et al*., 1990; Suns & Hitchin, 1990; Wren *et al*., 1991), dystrophy (dissolved organic carbon, total organic carbon, colour and Fe) (McMurtry *et al*., 1989; Swain & Helwig, 1989; Grieb *et al*., 1990; Wren *et al*., 1991; Fjeld & Rognerud, 1993) and hardness (Ca, Mg and conductivity) (Wren & MacCrimmon, 1983; McMurtry *et al*., 1989; Wren *et al*., 1991) have all been indicated as being significantly correlated with fish Hg concentrations.

A number of researchers have concluded that crayfish are effective indicators of Hg pollution in aquatic systems (Vermeer, 1972; Armstrong & Hamilton, 1973; Sheffy, 1978). They have also been demonstrated to be good indicators of Hg contamination in systems remote from direct Hg inputs (Allard & Stokes, 1989). Furthermore, concentrations of Hg in crayfish have shown a similar pattern to mercury levels in fish, mammals and sediments from the same areas (Sheffy, 1978; Wren *et al*., 1986; Parks, 1988; Allard & Stokes, 1989). Since crayfish are widely distributed and relatively large and long-lived, it would be advantageous to employ them as indicators of local mercury availability instead of using fish. Crayfish are relatively easy and inexpensive to collect, and their restricted range of movement could provide useful information in locating areas in an aquatic system where the availability of Hg to aquatic biota is relatively high.

When using an organism as an indicator of contamination, it is important that an appropriate tissue is analyzed. A number of researchers have concluded that crayfish abdominal muscle preferentially accumulates Hg (Johnels *et al*., 1967; Vermeer, 1972; Armstrong & Hamilton, 1973; Stinson & Eaton, 1983). However, Armstrong & Hamilton (1973) were the only ones to analyze multiple tissues. Since the crayfish analyzed by Armstrong & Hamilton (1973) were collected from an extensively Hg polluted water body, it is uncertain how applicable these results are to 'pristine' systems. Therefore, it is necessary to document Hg distribution in different tissues of crayfish from aquatic systems remote from anthropogenic sources of Hg before utilizing them as indicators of Hg contamination.

In addition to the lack of information on tissue distribution of total Hg in crayfish, there is little published data on methylmercury (CH_3Hg) levels in crayfish. Methylmercury is the form of Hg known to biomagnify in food chains, resulting in practically all of the Hg in fish muscle

tissue to be in the form of CH_3Hg (Grieb *et al*., 1990; Bloom, 1992). Crayfish are an important food source for wildlife, such as mink and otter (Linscombe *et al*., 1982; Toweill & Tabor, 1982), and several species of fish (Fedoruk, 1966; Reid, 1972; Stein, 1977). These crustaceans can occur in large numbers in a water body (Momot & Gowing, 1977; Momot, 1978; Reid & David, 1990) and could represent a significant pool of CH_3Hg that is potentially available to their consumers.

Our study had three objectives: (1) to examine total Hg concentrations in abdominal and other tissues of *Orconectes virilis* (Hagen), the most widely distributed Canadian crayfish species (Crocker & Barr, 1968; Momot, 1988), sampled from lakes distant from known anthropogenic sources of Hg; (2) to evaluate the relationship between total Hg concentrations in crayfish abdominal muscle and lake water chemical parameters; and (3) to determine the proportion of total Hg in crayfish abdomens that is in the form of CH_3Hg.

MATERIALS AND METHODS

Location and description of study lakes

Crayfish were collected from ten lakes located on the Precambrian Shield in south-central Ontario (Fig. 1). For comparative purposes, Hg levels were also examined in crayfish collected from Lake 224, located on the Precambrian Shield in the Experimental Lakes Area, northwestern Ontario. Further details of the study lakes can be found in Headon (1994).

All study lakes were remote from known sources of Hg contamination. Excluding water chemistry for Lake 224, which was obtained from I. Davies (Freshwater Institute, Winnipeg), and surface water Hg concentrations, water chemistry was provided by the Ontario Ministry of

the Environment and Energy (OMOEE) (Table 1; see below for methods). Cinder, Fawn, Fogal, Gullfeather and Hamer are mesotrophic lakes, while the remaining Ontario lakes are oligotrophic. All eleven lakes had low alkalinities ranging from 0.74 to 6.95 $mg.L^{-1}$. Lake water pH ranged from 5.53 to 7.02 and dissolved organic carbon (DOC) levels from 2.0 to 8.8 $mg.L^{-1}$.

Water chemistry

With the exception of Hamer, Blue Chalk and Lake 224, water samples were taken for general water chemistry within a week of crayfish collections for total Hg (Table 1). Excluding aluminum (Al), which was measured in April 1982, general water chemistry data for Lake 224 represents epilimnetic concentrations for July 1986. Detailed methods for lake water collections are outlined by Reid & David (1990) and Locke & Scott (1986). Since crayfish were collected on two different sampling dates from each of Big East, Blue Chalk, Crosson and Gullfeather, water chemistry reported in Table 1 for these lakes represents means for these dates.

Surface water (upper 0.5 m) total Hg concentrations for Lake 224 were obtained from I. Davies for December 1981. Water samples for the other study lakes, excluding Fogal due to its remote location, were collected and analyzed for total Hg in August 1992. Details of the methods used for bottle and sample preparation, and Hg analysis are described in Mierle (1990). Briefly, samples were collected in 1-litre borosilicate glass bottles with Teflon caps. For each lake, duplicate samples were taken from mid-lake, below surface, in front of a slowly moving row-boat. Upon collection, 5 mL of 40 mM HCl was added to the water sample to maintain

slightly acidic conditions and 4 mL of 50% hydrogen peroxide was added to maintain an oxidizing environment (Mierle, 1990). In the laboratory, water samples were UV-photooxidized and then preconcentrated by extraction with dithizone in trichlorobenzene, followed by back extraction into an acidic solution of potassium dichromate. Back extracts were then analyzed by cold vapour atomic absorption spectroscopy. All water samples were analyzed within a week of collection.

Field collections

With the exception of Lake 224 crayfish, which were provided by I. Davies, crayfish collections were conducted by the Dorset Research Centre, OMOEE. Sampling occurred during the months of June to September. Crayfish collections for CH_3Hg were conducted in 1992. With the exception of Fawn and Lake 224 crayfish, which were sampled in 1990 and 1986, respectively, crustaceans for total Hg analysis were collected in 1989.

Crayfish collections were made using modified wire-mesh minnow traps, each of which was baited with one perforated plastic film canister filled with commercial cat food. Traps were set late in the day and collected the following morning. Laboratory experiments revealed that minnow traps immersed for 24 h in 28 L of water (DOC 13.6 $mg.L^{-1}$; pH 6.0) with or without canisters of cat food, did not leach significant quantities of Hg (Headon, 1994).

All collected crayfish were identified to species and sexed according to Crocker & Barr (1968). Carapace length (CPL - distance from the rostral tip to the posteriomedian end of the cephalothorax) was measured using vernier calipers to the nearest 0.1 mm. Crustaceans were then brought back to the laboratory, placed individually in polyethylene bags and frozen until analysis.

Quality Assurance/Control of Crayfish Tissue and Hg Analyses

Analysis of total Hg was conducted in a laboratory at the Dorset Research Centre, OMOEE. Individual adult *O. virilis* were thawed, rinsed with distilled deionized water, patted dry with a damp cloth and weighed to the nearest 0.1 g. Individual whole tissues were dissected from each crayfish using stainless steel instruments and weighed to the nearest 0.1 mg. Eleven tissues were selected for the analysis of Hg: abdominal flexor muscle, extensor muscle, claw muscle (chela muscle), green glands, gills, digestive glands (hepatopancreas), mid and hind gut (intestine), gastric mill (stomach), eyes, carapace and tergum (exoskeleton that covers the dorsal surface of the abdomen). Tissue portions having a mass of 20 to 110 mg wet weight were placed in acid washed quartz or borosilicate glass test tubes. If the whole tissue was too large to be analyzed as one sample, a duplicate or triplicate analysis of the tissue was conducted.

The procedure used for the determination of total Hg was a modification of the method of Rasmussen *et al.* (1991). Each sample test tube received 2.0 mL of a 4:1 mixture of H_2SO_4 and HNO_3 and was heated on an aluminum hot block at 250°C for about 6 hours. Cooled samples were diluted with 8.0 mL of distilled deionized water and, prior to analysis, 1.5 mL of 10% hydroxylamine hydrochloride was added. Total Hg was determined for each sample by cold vapour atomic absorption spectroscopy. A 1.0 mL aliquot of the sample digest was dispensed into the degassing chamber, followed by 150 µL of 15% stannous chloride anhydrous dissolved in an aqueous solution of 10% HCl. Calibration standards (0, 0.1, 0.5 and 1.0 ng $Hg.mL^{-1}$) were analyzed throughout each run.

A typical run (47 samples) consisted of six procedural blanks, a triplicate of each of two standard reference materials, triplicate of white sucker muscle tissue (within lab quality

assurance test) and a 10 ng Hg spike to reagent blank in triplicate. All standard reference materials were dried to constant weight at 60°C. Between-run and within-run precision results for standard reference materials, white sucker and Hg spikes are listed in Table 2. The mean coefficient of variation (± SD) of Hg concentrations in triplicate samples of abdominal muscle was 4.8 ± 3.0% (n = 105). The mean observed Hg concentration for U S National Institute of Standards and Technology (NIST) citrus leaves No. 1572 was close to its certified value of 0.080 $\mu g.g^{-1}$ (Table 2). For NIST pine needles No. 1575, the mean Hg concentration obtained was a little low but within the certified concentration range (0.15 ± 0.05 $\mu g.g^{-1}$). The mean Hg concentration measured for National Research Council of Canada (NRCC) DOLT-1 (dogfish liver) was 12% higher than the upper certified 95% tolerance limit (0.225 ± 0.037 $\mu g.g^{-1}$).

Since visual peaks were not observed when procedural blanks were analyzed, the detection limit was determined by multiplying the height of the 'noise' of the base-line by three. A detection limit absorbance height of 6 mm was obtained. A sample weight, for example, of 100 mg (wet weight) had a detection limit of 0.004 $\mu g.g^{-1}$ and a 20 mg sample had a detection limit of 0.020 $\mu g.g^{-1}$. Three carapace samples were below detection.

All Hg determinations are based on and reported as wet weights. Conversion of wet weight concentrations to dry weight concentrations is made by dividing by the appropriate conversion factor. Calculated mean wet to dry conversion factors (± SD) were as follows: 0.23 ± 0.08 (n = 5) for green glands, 0.21 ± 0.02 (n = 12) for abdominal and extensor muscle, 0.21 ± 0.05 (n = 7) for claw muscle, 0.22 ± 0.03 (n = 7) for mid and hind gut, 0.28 ± 0.05 (n = 9) for digestive glands, 0.24 ± 0.02 (n = 7) for gastric mill, 0.14 ± 0.02 (n = 9) for gills, 0.29 ± 0.02 (n = 4) for eyes, 0.54 ± 0.06 (n = 8) for tergum and 0.57 ± 0.08 (n = 8) for carapace.

Portions of abdominal flexor muscle in adult male *O. virilis* collected for CH_3Hg analysis

were analyzed by Flett Research Ltd. (Winnipeg, Manitoba), following the procedure of Bloom (1989). The mean recovery of sample spikes was 107% with a standard deviation of 21% (n = 21).

Data analysis

For the 124 abdominal muscle data set, a Spearman rank correlation matrix was computed for the length-adjusted least squares geometric mean Hg concentrations and 12 lake chemical parameters [pH, alkalinity, conductivity, DOC, Al, Ca, Fe, Mg, sulphate (SO_4), total phosphorus, total Kjeldahl nitrogen and water Hg]. To maintain an overall significance of $P < 0.05$ for the number of comparisons made, correlations were considered significant at $P < 0.004$ (based on the Bonferroni procedure).

Total Hg was determined for multiple tissues in 35 male crayfish collected from six of the eleven study lakes. An analysis of variance (ANOVA) test was used to determine if Hg concentrations differed significantly among crayfish tissues within each lake. Only tissues with a sample size of at least three were compared. If Hg concentrations differed significantly among tissues within a lake ($P < 0.05$), the tissues were ranked based on their least squares geometric mean Hg concentrations and pairwise multiple comparisons were made using the Tukey HSD test.

Except for eyes (low sample size), each tissue type was compared among the six lakes. In addition, total Hg concentrations in abdominal flexor muscle of 124 crayfish (minimum of 10 from each lake) were compared among the eleven study lakes. Data for both sexes were pooled. However, only 18 of the 124 crayfish were females. An analysis of covariance (ANCOVA) with CPL as the covariate and lake as the treatment was performed separately on each tissue to test

whether Hg concentrations were dependent on crayfish CPL. If there was no significant interaction between lake and CPL ($P > 0.05$), homogeneity of slopes was assumed and an ANCOVA model without an interaction term was fitted to the data. When CPL did not explain a significant amount of the variation in Hg ($P > 0.05$), a regression on CPL was not required and an ANOVA was applied with lake as the factor. Pairwise multiple comparisons were made with the Tukey HSD test if Hg concentrations in a tissue differed significantly ($P < 0.05$) among the lakes. However, if CPL was indicated as being a significant predictor of Hg concentrations in a tissue, size-corrected means Hg concentrations were compared among the lakes by performing the Tukey HSD test on the estimated ANCOVA model.

Data were analyzed statistically using SYSTAT for Windows, version 5 (Wilkinson, 1992). Tissue Hg concentrations and CPLs were both $\log_{10}$-transformed to satisfy the requirements of the statistical tests.

RESULTS

Abdominal muscle

Total Hg concentrations in abdominal flexor muscle of 124 crayfish sampled from eleven lakes ranged from 0.035 to 0.719 $\mu g.g^{-1}$ for crayfish having a CPL which ranged from 21.5 to 48.4 mm (Table 3). There was no significant interaction between covariate (CPL) and factor (lake) when an ANCOVA test was performed ($P = 0.08$). When the interaction term was omitted, CPL was a significant predictor of Hg concentrations ($P < 0.001$). Total Hg concentrations in abdominal muscle adjusted for CPL were significantly different among the eleven lakes ($P < 0.001$). The Tukey HSD test revealed a number of overlapping similarities in

Hg levels (Table 4). An overall general conclusion was that Blue Chalk, Clear and Lake 224 had the three lowest size-adjusted mean Hg concentrations in abdominal muscle and Big East, Fogal and Hamer had the three highest.

Multiple tissues

Tissue Hg concentrations are summarized in Table 5. The range in arithmetic mean Hg concentrations across the six study lakes for each tissue was as follows: green glands 0.115 to 0.261, muscle tissue 0.065 to 0.144, mid and hind gut 0.039 to 0.058, digestive glands 0.031 to 0.060, gastric mill 0.024 to 0.035, gills 0.018 to 0.030 (outlier value for Lake 224 of 0.055), eyes 0.024 to 0.034, tergum 0.014 to 0.031 and carapace 0.009 to 0.014 $\mu g.g^{-1}$. With the exception of Fawn Lake, CPLs were fairly similar among the lakes (Table 5). Crayfish from Fawn Lake had a mean CPL which was approximately 10 mm greater than the means for each of the other lakes. It should also be noted that crayfish from Lake 224 were relatively small compared with many of the other crustaceans.

Within each lake, Hg concentrations differed significantly among tissues ($P < 0.001$). Since a number of overlapping similarities in tissue Hg levels were revealed with the Tukey HSD test, definite conclusions cannot be made (Table 5). In general, green glands and muscle tissue (abdominal and extensor) had the highest Hg concentrations while exoskeleton (carapace) samples had the lowest. The typical relationship found among tissues within each lake was green glands ≥ abdominal muscle = extensor muscle = claw muscle > mid and hind gut = digestive glands ≥ gastric mill = gills ≥ tergum ≥carapace. One notable exception to this pattern was gill tissue (0.055 μg.g) for Lake 224 crayfish whose Hg concentration was not significantly different from that of muscle tissue.

For each tissue compared across the six study lakes (Table 5), there was no significant interaction between covariate (CPL) and factor (lake) ($P \geq 0.1$). ANCOVA tests performed on models without interactions revealed that for all tissues except abdominal flexor muscle ($P = 0.002$) and claw muscle ($P = 0.003$), CPL did not explain a significant amount of the variation in tissue Hg concentrations ($P > 0.1$). Total Hg concentrations in abdominal and claw muscle adjusted for CPL were significantly different among the six lakes ($P \leq 0.01$). Significant among lake differences were also found for green glands, gills, digestive glands, mid and hind gut and tergum ($P < 0.005$). There were no among lake differences in Hg concentrations for gastric mill, carapace and extensor muscle ($P \geq 0.1$). Extensor muscle from Fawn Lake was not included in the comparison because of the small sample size ($n = 2$).

Tukey HSD results are displayed in Table 6 and Table 7 for those tissues for which there were significant among lake differences in Hg concentrations. For claw muscle, the Tukey test failed to detect differences in length-adjusted Hg concentrations even though the ANCOVA test indicated significant among lake differences (Table 7). Since there were a number of overlapping similarities in tissue Hg levels among lakes, definite conclusions cannot be made. The only consistent pattern was that Blue Chalk crayfish had significantly lower Hg concentrations in all tissues, except for green glands, than Big East (Table 6). One other notable finding was that Hg concentrations in gill tissue of Lake 224 crayfish were significantly greater than in gill tissue of crayfish from each of the other lakes

Lake water chemical parametres

Lake chemical variables that were significantly correlated with size-adjusted mean Hg concentrations in crayfish abdominal flexor muscle were pH, DOC, Fe, Al and water Hg (Table 8). The Spearman correlation coefficients were positive for DOC, Fe, Al and water Hg, and negative for pH. It should be noted that Hg in water was highly correlated with each of the other four parameters (r_s = 0.838, -0.843, 0.870 and 0.902 for DOC, pH, Al and Fe, respectively). In addition, Al was highly correlated with pH (r_s = -0.932), DOC (r_s = 0.849) and Fe (r_s = 0.837), and Fe was highly correlated with pH (r_s = -0.811) and DOC (r_s = 0.963). Since values for the water chemical parameters Fe and Hg were not available for Fawn and Fogal, respectively, these lakes were deleted from correlations involving the respective parameter. In addition, Lake 224 water Hg concentrations were not included in the analysis due to the large range in values reported for this parameter (Table 1).

Lake chemical variables representative of productivity (total phosphorus and total Kjeldahl nitrogen) were positively correlated with crayfish Hg concentrations (r_s = 0.744 and r_s = 0.724, respectively). However, these correlations were not significant at α= 0.05. Likewise, there was a fairly strong, but insignificant, relationship with alkalinity (r_s = -0.674).

Methylmercury

Both total and CH_3Hg concentrations were measured in abdominal flexor muscle of 20 male crayfish sampled from Blue Chalk, Clear, Fawn and Hamer in 1992 (Table 9). Crayfish having a similar CPL (range: 34 to 39 mm) were sampled from each lake to avoid the inclusion of a size effect. Methylmercury accounted for most (100%) of the total Hg in crayfish muscle tissue. Unfortunately, a very large standard deviation is associated with this value (± 32%).

DISCUSSION

Tissue levels

The tissue distribution pattern of total Hg observed in our study closely resembles that reported by Armstrong & Hamilton (1973) for *O. virilis* sampled from the heavily Hg polluted Clay Lake, northwestern Ontario. The pattern is similar even though the Hg concentrations differ by as much as two orders of magnitude. Abdominal muscle of Clay Lake crayfish had a mean Hg concentration of approximately 10 $\mu g.g^{-1}$ wet weight and, for other organs analyzed, levels ranged from 0.9 to 6.0 $\mu g.g^{-1}$ (Armstrong & Hamilton, 1973). Other organs included (in descending order with respect to Hg concentration) heart, claw muscle, green gland, intestine, digestive glands, gastric mill, gills, carapace and head.

Three additional studies have compared Hg levels in crayfish abdominal muscle with other tissues. Johnels *et al.* (1967) documented total Hg concentrations ($\mu g.g^{-1}$ wet weight) for carapace (0.027), digestive glands (0.090) and abdominal muscle (0.140) in crayfish (*Astacus fluviatilis*) from aquatic systems in Sweden that are comparable to values obtained in this study (sample size unknown). For *O. virilis* sampled from a slightly contaminated river in Manitoba, Vermeer (1972) noted that abdominal muscle contained about three times more Hg (0.078 $\mu g.g^{-1}$ wet weight) than the remaining collective parts (0.027 $\mu g.g^{-1}$). Likewise, Stinson & Eaton (1983) found that abdominal muscle of commercially caught crayfish (*Pacifasticus leniusculus*) contained substantially higher concentrations of Hg than either exoskeleton (included gill tissue) or viscera.

Mercury concentrations determined in our study for abdominal muscle fall within the range of reported values for crayfish from other 'pristine' water bodies (France, 1987; Wren & Stokes, 1988; Allard & Stokes, 1989). For example, total Hg concentrations in abdominal muscle of seven crayfish species from 13 remote lakes located in the Algonquin Region of south-central Ontario ranged from 0.021 to 0.614 $\mu g.g^{-1}$ wet weight, while crayfish whole-body weights ranged from 0.4 to 35.0 g (Allard & Stokes, 1989). Mercury levels reported in this paper for abdominal muscle ranged from 0.035 to 0.719 $\mu g.g^{-1}$ for crayfish having a mass of 1.6 to 22.8 g. These values also fall within the range of concentrations measured in *O. virilis* from contaminated systems (Vermeer, 1972; Sheffy, 1978; Munro & Gummer, 1980). For example, *O. virilis* sampled from along the Hg contaminated Wisconsin River had total Hg concentrations in their abdominal muscle that ranged from 0.07 to 0.56 $\mu g.g^{-1}$ wet weight (Sheffy, 1978). Many of these crayfish were considerably larger than the ones analyzed in this study.

Statistical analysis of the data revealed that CPL was a significant predictor of Hg concentrations in abdominal muscle and claw muscle. Mercury bioconcentration (increase in Hg concentration with length of exposure) has been well demonstrated in fish through correlations between Hg concentration and size (McMurtry *et al.*, 1989; Grieb *et al.*, 1990; Wren *et al.*, 1991). Positive correlations between Hg concentrations in crayfish abdominal muscle and whole-body weights have also been reported (Armstrong & Hamilton, 1973; Stinson & Eaton, 1983; Allard & Stokes, 1989). The fact that CPL did not explain a significant amount of the variation in Hg in tissues other than the two muscle tissues could have been the result of an insufficient sample size, too small a size range or a real lack of correlation between crayfish size and Hg concentration. A tissue that does not bioconcentrate Hg may have an excretory role for Hg, or perhaps Hg resides in the tissue only temporarily until it is assimilated by another tissue.

The form of Hg (methyl or inorganic) that is preferentially accumulated by a tissue must be considered since it could greatly influence Hg dynamics in that tissue.

The only studies which could be located that examined CH_3Hg levels in freshwater decapods were those by Armstrong & Hamilton (1973) and Hildebrand *et al.* (1980). For the claw muscle of one crayfish specimen from the Hg contaminated Clay Lake, 90% of the total Hg was in the form of CH_3Hg (Armstrong & Hamilton, 1973). Decapoda collected from the Hg contaminated North Fork Holston River, Virginia, had mean percentages of CH_3Hg (for whole-body) ranging from 48 to 80% (Hildebrand *et al.*, 1980). Although a sizable range in CH_3Hg percentages was acquired in our study, it appears that CH_3Hg accounts for a large portion of the total Hg in crayfish abdominal flexor muscle.

Mercury localization

For crayfish, the relative importance of Hg uptake from water versus food is uncertain. Parks *et al.* (1988) concluded that food was the most important route of Hg uptake for caged crayfish held *in situ*. However, this conclusion is questionable since the study crayfish were fed fish tissue having a Hg concentration of 0.13 $\mu g.g^{-1}$, a value which is likely greater than that in foods normally consumed. Therefore, both routes of entry will be considered during the following discussion.

Andersen & Baatrup (1988) and Chang *et al.* (1983) examined Hg distribution in crustaceans that were exposed to *waterborne* radiolabelled mercuric chloride ($^{203}HgCl_2$). The rank order of Hg concentrations ($cpm.g^{-1}$ wet weight) in tissues excised from *O. virilis* exposed to waterborne ^{203}Hg for 35 days was: green glands > hepatopancreas (digestive glands) > gills > gut > carapace > ovary > abdominal muscle (Chang *et al.*, 1983). Likewise, the hepatopancreas

and gill tissue of the marine brown shrimp (*Crangon crangon*) contained substantially more assimilated ^{203}Hg than abdominal tissue after 14 days of exposure (Andersen & Baatrup, 1988). For the first three days most of the assimilated ^{203}Hg was located in the gills. However, between day four and the termination of the study (day 14) highest ^{203}Hg concentrations were in the hepatopancreas followed by gills (Andersen & Baatrup, 1988). Additional evidence that suggests that crustaceans are able to transport inorganic Hg from gill tissue to the hepatopancreas was provided by Vernberg & O'Hara (1972) when they exposed fiddler crabs (*Uca pugilator*) to waterborne inorganic Hg. If waterborne Hg accumulated by gill tissue can be transported to the hepatopancreas, this would explain why Chang *et al.* (1983) obtained relatively high concentrations of ^{203}Hg in this tissue.

It was suggested by Chang *et al.* (1983) that crayfish hepatopancreas and gill tissue should be selected as target organs for assessing metal accumulation, including Hg, in crayfish. Hepatopancreas, digestive gut and gill tissue of natural populations of crayfish have been indicated to be the primary target tissues of Cd, Cu, Zn and Pb deposition (Anderson & Brower, 1978; Bagatto & Alikhan, 1987*a*, *b*, *c*; Keenan & Alikhan 1991; Madigosky *et al.*, 1991). However, based on our results, as well as those of Armstrong & Hamilton (1973), hepatopancreas and gill tissue are not the primary sites of Hg accumulation in natural crayfish populations, even in remote systems. Therefore, we do not recommend that these tissues are used to assess Hg bioavailability.

We believe that the most probable explanation for the difference in the findings of the above ^{203}Hg studies and our study was that Andersen & Baatrup (1988) and Chang *et al.* (1983) exposed their study organisms to *inorganic* Hg and the primary route of exposure was through water. Recently, Headon (1996) determined that abdominal muscle of *O. virilis*, which had been

fed food labelled with $CH_3{}^{203}HgCl$ and allowed to depurate for 52 to 148 days, contained the highest concentration of ^{203}Hg. All other tissues, including gill and hepatopancreas, had relatively low ^{203}Hg concentrations. Saouter *et al.* (1993) observed that the distribution of Hg in burrowing mayflies (*Hexagenia rigida*) exposed to radiolabelled Hg for nine days depended on the route of exposure (sediment or water) and the form of Hg (methyl or inorganic). When the water column of the experimental units was labelled with inorganic Hg, gills contained 49% and gut 8% of the total whole-body ^{203}Hg burden after nine days of exposure. In contrast, gills contained 20% and guts 17% of the ^{203}Hg burden when the water was labelled with $CH_3{}^{203}Hg$. Tissue accumulation results were quite different for labelled sediments. For added ^{203}Hg and $CH_3{}^{203}Hg$, purged guts contained 43% and 18%, respectively, of the whole-body burden. For both forms of ^{203}Hg, gills contained only about 5% of the total accumulated ^{203}Hg.

In the laboratory, muscle tissue in fish has been shown to accumulate the greatest burdens of CH_3Hg (Giblin & Massaro, 1973; Pentreath, 1976; deFreitas, 1977; Boudou & Ribeyre, 1983; Boudou & Ribeyre, 1985). In contrast, ingested inorganic Hg appears to accumulate mainly in the intestinal tract of fish with very little being distributed to other tissues (Pentreath, 1976; deFreitas, 1977; Boudou & Ribeyre, 1985). For example, after a 35 to 40 day depuration period, Pentreath (1976) found that the majority of the retained ^{203}Hg originally assimilated from $^{203}HgCl_2$ labelled food was associated with the gut wall of plaice (*Pleuronectes platessa* L.). Less than 5% was located in the muscle tissue. Conversely, for ^{203}Hg assimilated from $CH_3{}^{203}HgCl$ labelled food, less than 5% of the whole-body ^{203}Hg burden retained at the end of the depuration period was associated with the gut. The muscle tissue contained about 80% of the burden (Pentreath, 1976). As with ingested Hg, the distribution of Hg in fish that have accumulated Hg directly from water differs with the form of Hg (Boudou & Ribeyre, 1983).

Experiments which focused on the depuration of Hg assimilated from water contaminated with $HgCl_2$ revealed that over a 250 day depuration period there was a very rapid decrease in the Hg burden of gills, a gradual increase for kidneys and a very slow increase for muscle tissue (contained 8% of the total Hg on day zero and 21% on day 250) (Boudou & Ribeyre, 1983). For CH_3HgCl, there was also a rapid decrease in gill content. However, there was a large increase in muscle Hg content (contained 21% of the total Hg on day zero and 86% of the remaining Hg on day 250) (Boudou & Ribeyre, 1983).

For each of the six study lakes (Table 6), the mean Hg concentration in green glands was either significantly greater or not significantly different from the mean Hg concentration in muscle tissue. Chang *et al.* (1983) also found that these glands contained concentrations of ^{203}Hg that were higher than those in each of the other tissues analyzed. Green glands are excretory organs through which the majority of any excess water in crayfish is eliminated as urine (Holdich & Reeve, 1988). Green glands could act as an excretory organ for Hg, as well as a site of storage. Microscopic examination of the green glands of marine brown shrimp exposed to waterborne $^{203}HgCl_2$ revealed that extensive amounts of Hg accumulation had taken place (Andersen & Baatrup, 1988). A heavy metal binding protein, perhaps metallothionein, capable of binding to inorganic Hg in green glands of crayfish (*Pasifastacus leniusculus*) has been observed (Ellis & Fuller, 1979). It should be noted that green glands excised from the 35 study crayfish were extremely small (15 - 50 mg), unlike abdominal muscle, for example, which had a mass ranging from 260 to 1770 mg. Therefore, green glands account for relatively little of the whole-body Hg burden.

Total Hg concentrations in gastric mill were often significantly less than they were in digestive glands and mid and hind gut. Mercury levels found in gastric mill and gut are partly a

reflection of unassimilated Hg and should not be regarded as representing Hg accumulated over time. Mercury concentrations determined for exoskeleton tissue may have been primarily the result of Hg adsorption. Since mature male *O. virilis* moult twice a year (Berrill, 1978), any Hg associated with the exoskeleton at this time would have been lost. For some of the study lakes, tergum had a mean Hg concentration that was significantly greater than carapace. This could have been due to the close association of tergum to abdominal muscle. Perhaps small quantities of muscle tissue still attached to the tergum surface inflated the Hg levels.

Among lake differences in tissue concentrations

The most pronounced among lake difference with respect to tissue Hg concentrations was for gill. Mercury concentrations in Lake 224 crayfish gill were significantly greater than for crayfish sampled from each of the Muskoka-Haliburton lakes. Relatively high gill Hg levels may indicate significant Hg uptake from water. It is also possible that physiological differences between the crayfish population in Lake 224 and the populations in the Muskoka-Haliburton lakes could explain observed differences in Hg tissue distribution.

There was a significant difference in size-corrected Hg concentrations in abdominal flexor muscle among the eleven study lakes. Crayfish Hg concentrations were significantly positively correlated with the lake chemical parameters DOC, Fe, Al and Hg. There was a significant negative correlation between lake pH and muscle Hg concentrations. It is uncertain which of these parameters are influencing Hg levels in the crayfish since they were all highly intercorrelated. Aluminum, Fe, DOC and pH have been identified to be significantly correlated with Hg concentrations in fish (McMurtry *et al.*, 1989; Swain & Helwig, 1989; Grieb *et al.*,

1990; Suns & Hitchin, 1990; Wren *et al.*, 1991). France (1987) observed that the mean Hg concentration for crayfish abdominal sections was approximately twice as high for an acidic lake as for three circumneutral lakes. Chemical parameters representative of lake acidity (pH and alkalinity) and hardness (Ca, Mg and conductivity) were all significantly negatively correlated with abdominal muscle Hg concentrations of crayfish collected from lakes in the Algonquin Region (Allard & Stokes, 1989). Conductivity was found to explain 54% of the among lake variation in Hg concentrations (Allard & Stokes, 1989). For this study, conductivity, Mg and Ca were very weakly correlated with crayfish Hg concentrations. In addition, unlike Allard & Stokes (1989), we observed a significant positive correlation between Hg concentrations and DOC.

It has been suggested through detailed monitoring of Hg levels in catchment run-off that observed positive correlations between DOC and Hg in fish may be due to the transportation from catchments of Hg in association with refractory organic substances, specifically humic compounds (Lee & Hultberg, 1990; Mierle & Ingram, 1991). The ability of humic substances to complex with Hg has been established in laboratory studies (Lodenius *et al.*, 1983; Thanabalasingam & Pickering, 1985). Miskimmin *et al.*, (1992) demonstrated decreased net CH_3Hg production in lake water with increasing DOC levels. This led them to hypothesize that elevated fish Hg concentrations observed in highly coloured lakes resulted from allochthonous inputs of CH_3Hg associated with organics rather than direct CH_3Hg formation within the lake.

There have been a number of hypotheses put forward to explain the often observed negative correlation between Hg in biota and water pH (Richman *et al.*, 1988; Winfrey & Rudd, 1990; Gilmour & Henry, 1991). Possibilities include, for example, the stimulation of net microbial methylation, the increase in the availability of inorganic Hg (Hg^{2+}) for methylation and

the alteration of trophic structure in the acidified system. An increase in net CH_3Hg formation with decreasing water pH has been shown to occur in lake water (Xun *et al.,* 1987; Miskimmin *et al.*, 1992) and at the sediment-water interface (Xun *et al*., 1987). Miskimmin *et al.* (1992) hypothesized that high Hg levels in fish from acidic lakes could be due in part to an increase in Hg^{2+} availability for methylation (reduced Hg^{2+} binding by DOC as pH is lowered). Miller & Akagi (1979) found that pH did not affect the amount of CH_3Hg formed in the sediment but it did affect how it was partitioned. A decrease in pH resulted in an increase in the quantity of CH_3Hg in the water column.

CONCLUSION

Our results indicate that crayfish abdominal muscle may be a good indicator of Hg bioavailability in freshwater systems provided that crayfish within the same size range are analyzed. Abdominal muscle appears to be a more reliable indicator of Hg bioavailability than any other crayfish tissue. Both green glands and muscle tissue preferentially concentrate Hg. However, we recommend that muscle tissue rather than green glands is utilized to assess Hg accumulation in crayfish. Green glands could act as both an excretory organ and a site of storage of Hg. Furthermore, it is plausible that the majority of Hg in green glands is inorganic and, therefore, does not accurately reflect the availability of Hg in the surrounding environment. We further suggest, due to its mass and ease of excision, that abdominal flexor muscle be the choice of muscle tissue.

Many of the crayfish analyzed for Hg had concentrations below the Canadian 0.5 $\mu g.g^{-1}$ wet weight upper limit for safe fish consumption, although some large crayfish from Hamer

Lake had Hg concentrations close to this limit. Since it appears that a large portion of the Hg concentrated in abdominal muscle is in the form of CH_3Hg, crayfish inhabiting remote lakes in south-central Ontario could represent a significant source of CH_3Hg to their consumers. Abdominal muscle Hg concentrations were shown to be significantly correlated to the same chemical parameters which have been demonstrated to explain significant amounts of among lake variation in fish Hg concentrations. This suggests that crayfish abdominal muscle provides a good estimate of the availability of CH_3Hg within an aquatic system to fish, and ultimately wildlife and humans.

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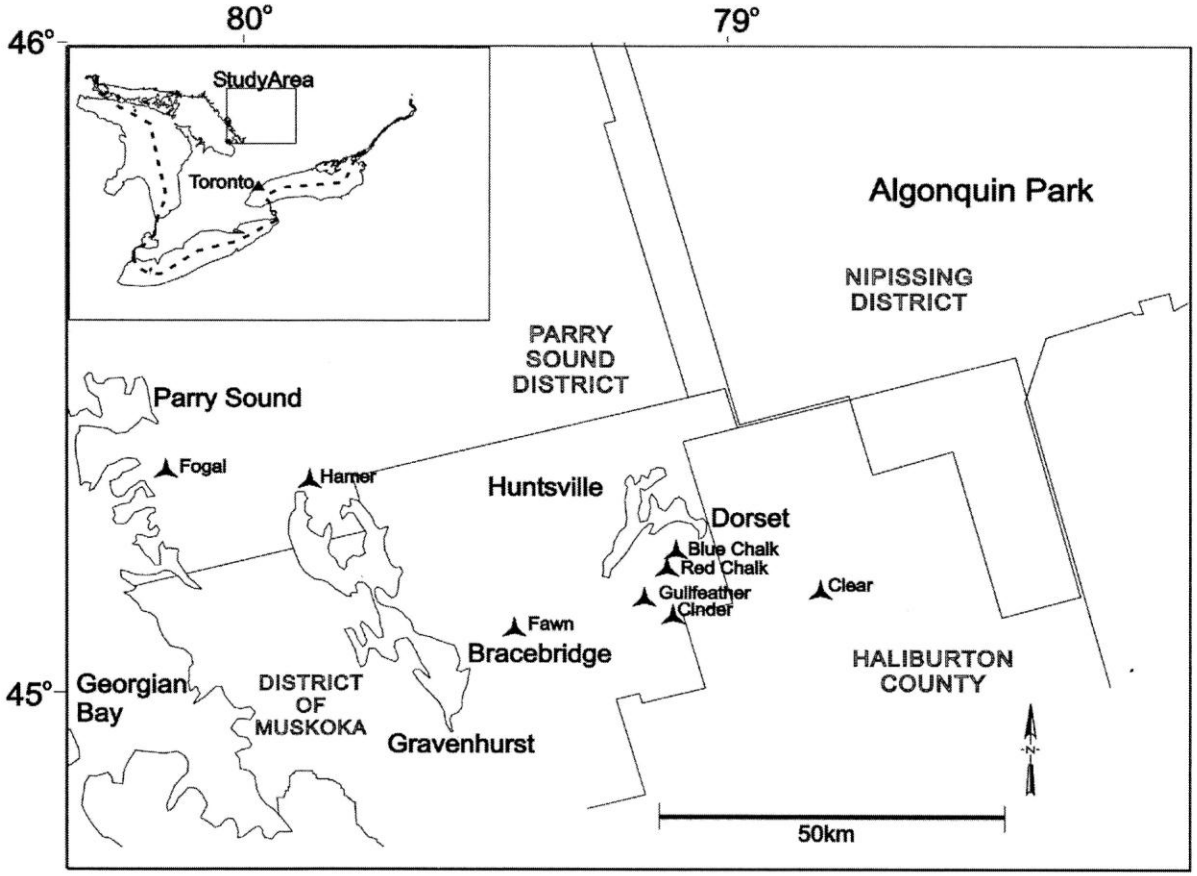

Figure 1: Location of Study Lakes

Table 1. Water chemistry for the study lakes

	Big East	Blue Chalk	Cinder	Clear	Crosson	Fawn	Fogal	Gull-feather	Hamer	Lake 224	Red Chalk
pH	6.31	6.78	6.09	6.52	5.90	6.67	5.73	5.98	5.53	7.02	6.64
Alkalinity ($mg.L^{-1}$)[a]	1.94	4.14	1.76	2.39	0.74	5.1	1.53	1.11	1.62	6.95	3.33
Conductivity ($\mu S.cm^{-1}$)	52.6	28.4	26.1	28.2	25.9	35.1	23.1	29.0	26.3	23	28.7
DOC ($mg.L^{-1}$)	4.9	2.0	5.0	2.1	4.3	7.9	8.8	5.1	8.7	2.8	2.7
Al ($\mu g.L^{-1}$)	21	2	38	4	40	27	117	40	170	2	7
Ca ($mg.L^{-1}$)	3.18	2.63	2.30	2.85	2.33	3.31	1.90	2.83	2.50	1.83	2.65
Fe ($mg.L^{-1}$)	0.10	0.01	0.13	0.02	0.04	n.a.[b]	0.46	0.28	0.82	<0.04	0.01
Mg ($mg.L^{-1}$)	0.90	0.76	0.73	0.61	0.64	0.94	0.60	0.79	0.54	0.47	0.83
SO_4 ($mg.L^{-1}$)	7.10	6.60	6.55	7.70	7.65	6.7	5.15	8.00	4.95	3.40	7.00
Total P ($\mu g.L^{-1}$)	7.8	4.6	13.5	4.6	7.0	12.9	16.5	10.0	13.3	2.0	3.7
Total Kjeldahl N ($\mu g.L^{-1}$)	300	170	310	210	280	450	510	325	420	265	160
Hg ($ng.L^{-1}$)	2.21	0.56	1.35	0.69	0.97	1.73	n.a.	1.25	1.74	<1 - 6[c]	0.72

[a]Total inflection point alkalinity.
[b]Data not available.
[c]Range of three samples.

Table 2. Between-run and mean within-run precision of triplicates of NIST and NRCC reference material, white sucker and 10 ng Hg spikes to blanks. Concentrations of certified reference material are in parentheses.

Material (Certified Concentration)	n (runs)	Mean Hg ± SD ($\mu g.g^{-1}$)	Between-run CV (%)[a]	Within-run CV (%)
NIST Pine No. 1575 (0.15 ± -0.05 $ug.g^{-1}$)	28	0.119 ± 0.006	5.3	4.4
NIST Citrus No. 1572 (0.080 $ug.g^{-1}$)	30	0.082 ± 0.006	7.0	6.4
NRCC DOLT-1[b] (0.225 ± -0.037 $ug.g^{-1}$)	22	0.294 ± 0.019	6.4	3.8
White sucker	33	0.218 ± 0.010	4.4	3.6
Spike (10 ng)	32	9.98 ± 0.33	3.3	1.8

[a]Coefficient of variation.
[b]Duplicates only were analyzed for 10 of the 22 runs.

Table 3. Arithmetic mean carapace lengths and abdominal flexor muscle Hg concentrations of *O. virilis* collected from the eleven study lakes (ranges in parentheses)

		CPL (mm)		Hg ($\mu g.g^{-1}$ wet wt.)	
Lake	n	Mean	SE	Mean	SE
Big East	16	30.5 (26.5 - 36.9)	0.8	0.137 (0.055 - 0.238)	0.011
Blue Chalk	14	32.0 (26.4 - 36.2)	0.8	0.071 (0.035 - 0.116)	0.006
Cinder	12	32.2 (28.0 - 39.0)	0.9	0.136 (0.082 - 0.229)	0.012
Clear	10	32.6 (31.0 - 34.9)	0.5	0.077 (0.050 - 0.139)	0.008
Crosson	11	29.0 (26.2 - 33.5)	0.7	0.102 (0.073 - 0.130)	0.006
Fawn	10	40.6 (38.3 - 45.2)	0.7	0.146 (0.119 - 0.228)	0.011
Fogal	10	35.8 (31.5 - 38.8)	0.9	0.226 (0.146 - 0.288)	0.014
Gullfeather	10	26.7 (21.5 - 29.1)	0.8	0.096 (0.071 - 0.132)	0.006
Hamer	10	43.2 (35.7 - 48.4)	1.3	0.404 (0.187 - 0.719)	0.049
Lake 224	11	26.2 (22.8 - 29.0)	0.6	0.066 (0.045 - 0.112)	0.007
Red Chalk	10	33.2 (27.8 - 38.0)	1.0	0.106 (0.043 - 0.146)	0.011

Table 4. Carapace length-adjusted least squares geometric mean Hg concentrations (µg.g^{-1} wet weight) for abdominal muscle. Concentrations connected with the same line are not significantly different (*P* > 0.05; Tukey HSD)

Lake	Hg
Blue Chalk	0.069
Clear	0.072
Lake 224	0.092
Fawn	0.095
Red Chalk	0.095
Crosson	0.121
Cinder	0.132
Gullfeather	0.133
Big East	0.144
Fogal	0.186
Hamer	0.227

Table 5. Arithmetic mean tissue Hg concentrations (µg.g^{-1} wet weight) (± SE) for male crayfish from six of the study lakes (sample size in parentheses). Within each column, Hg concentrations followed by the same letter are not significantly different ($P > 0.05$; Tukey HSD)

Tissue	Big East	Blue Chalk	Clear	Crosson	Fawn	Lake 224
Green glands	0.150 ± 0.010 (5) a	0.162 ± 0.010 (5) a	0.231 ± 0.028 (5) a	0.148 ± 0.012 (7) a	0.115 ± 0.015 (3) a	0.261 ± 0.041 (3) a
Abdominal muscle	0.144 ± 0.010 (7) a	0.069 ± 0.008 (6) b	0.091 ± 0.013 (5) b	0.095 ± 0.008 (7) b	0.127 ± 0.006 (3) a	0.073 ± 0.009 (7) b
Extensor muscle	0.111 ± 0.011 (4) a,b	0.065 ± 0.007 (5) b	0.081 ± 0.020 (3) b	0.092 ± 0.010 (5) b	0.102, 0.143 (2)	0.100 ± 0.022 (3) b
Claw muscle	0.102 ± 0.010 (6) b	0.067 ± 0.012 (6) b	0.086 ± 0.016 (5) b	0.110 ± 0.012 (5) a,b	0.088 ± 0.009 (3) a	0.087 ± 0.021 (5) b
Mid and hind gut	0.053 ± 0.004 (5) c	0.039 ± 0.002 (5) b,c	0.052 ± 0.005 (5) b,c	0.058 ± 0.002 (7) c	0.045 ± 0.002 (3) b	0.049 ± 0.002 (3) b,c
Digestive glands	0.060 ± 0.004 (5) c	0.031 ± 0.005 (5) c,d	0.032 ± 0.002 (5) c,d	0.054 ± 0.006 (7) c,d	0.048 ± 0.004 (3) b	0.048 ± 0.013 (5) b,c
Gastric mill	0.024 ± 0.002 (5) d	0.025 ± 0.002 (5) c,d	0.025 ± 0.004 (5) d,e	0.028 ± 0.001 (7) e	0.031 ± 0.002 (3) b,c	0.035 ± 0.008 (5) c,d
Gills	0.027 ± 0.001 (5) d	0.018 ± 0.002 (5) d,e	0.024 ± 0.002 (5) d,e	0.030 ± 0.002 (7) e	0.021 ± 0.001 (3) c	0.055 ± 0.004 (5) b,c
Eyes	-	0.017, 0.031 (2)	-	0.034 ± 0.004 (3) d,e	0.028, 0.031 (2)	-
Tergum	0.025 ± 0.002 (5) d	0.014 ± 0.002 (5) e	0.015 ± 0.002 (5) e,f	0.024 ± 0.002 (6) e	0.031 ± 0.007 (3) b,c	0.019 ± 0.003 (5) d,e
Carapace	0.014 ± 0.001 (5) e	0.009 ± 0.002 (3)[1] e	0.009 ± 0.002 (5) f	0.013 ± 0.002 (6) f	0.012 ± 0.001 (3) d	0.014 ± 0.002 (4)[2] e
CPL (mm)	32.8 ± 0.9 (28.8 - 36.9)[3]	30.8 ± 1.5 (26.4 - 36.2)	32.9 ± 0.8 (31.1 - 34.9)	30.1 ± 0.9 (26.8 - 33.5)	40.5 ± 1.1 (38.3 - 41.6)	27.2 ± 0.7 (24.0 - 29.0)

[1]Does not include two samples which were below the detection limit (detection limits were 0.009 and 0.010 µg.g^{-1}).

[2]Does not include a sample which was below the detection limit (detection limit was 0.011 µg.g^{-1}).

[3]Range.

Table 6. Least squares geometric mean Hg concentrations (µg.g^{-1} wet weight) for tissues for which there were significant among lake differences in Hg concentrations (ANOVA; *P* < 0.005). Concentrations connected with the same line are not significantly different (*P* > 0.05; Tukey HSD)

Tissue							
Green glands	Lake	Fawn	Crosson	Big East	Blue Chalk	Clear	Lake 224
	Hg	0.114	0.145	0.149	0.161	0.223	0.255
Gills	Lake	Blue Chalk	Fawn	Clear	Big East	Crosson	Lake 224
	Hg	0.018	0.021	0.023	0.027	0.029	0.054
Digestive glands	Lake	Blue Chalk	Clear	Lake 224	Fawn	Crosson	Big East
	Hg	0.030	0.031	0.042	0.048	0.052	0.060
Mid and hind gut	Lake	Blue Chalk	Fawn	Lake 224	Clear	Big East	Crosson
	Hg	0.038	0.045	0.048	0.051	0.053	0.058
Tergum	Lake	Blue Chalk	Clear	Lake 224	Crosson	Big East	Fawn
	Hg	0.013	0.014	0.018	0.023	0.025	0.029

Table 7. Carapace length-adjusted least squares geometric mean Hg concentrations (µg.g^{-1} wet weight) for abdominal muscle and claw muscle. Concentrations connected with the same line are not significantly different (*P* > 0.05; Tukey HSD)

Tissue							
Abdominal muscle	Lake	Blue Chalk	Clear	Fawn	Lake 224	Crosson	Big East
	Hg	0.069	0.080	0.080	0.090	0.100	0.130
Claw muscle	Lake	Fawn	Blue Chalk	Clear	Big East	Crosson	Lake 224
	Hg	0.043	0.064	0.070	0.094	0.122	0.126

Table 8. Spearman rank correlation coefficients for length-adjusted least squares geometric mean abdominal muscle Hg concentrations and twelve chemical water parameters, *P < 0.004

Lake variable	n	Spearman rank correlation coefficient
pH	11	-0.843*
Alkalinity	11	-0.674
DOC	11	0.838*
Fe	10	0.902*
Ca	11	-0.128
Mg	11	-0.091
Conductivity	11	-0.009
Total Kjeldahl N	11	0.724
Total P	11	0.744
SO_4	11	-0.141
Al	11	0.870*
Hg	9	0.837*

Table 9. Mean (± SE) carapace lengths and abdominal flexor muscle CH_3Hg and total Hg concentrations ($\mu g.g^{-1}$ wet weight) for *O. virilis* collected from four of the eleven study lakes (five individuals per lake)

Lake	CPL (mm)	CH_3Hg	Total Hg	% CH_3Hg
Blue Chalk	35.5 ± 0.6	0.080 ± 0.011	0.066 ± 0.005	122 ± 17
Clear	35.7 ± 0.5	0.060 ± 0.012	0.073 ± 0.007	83 ± 14
Fawn	37.0 ± 0.7	0.144 ± 0.021	0.152 ± 0.011	94 ± 11
Hamer	37.0 ± 0.8	0.211 ± 0.022	0.211 ± 0.015	101 ± 13